The Southern blot banding patterns (Fig. 3A) were identical for MCC347 and its metastasis, MCC348, in line with 3′-RACE results (Fig. 1B) and confirming that MCC348 arose as a metastatic clone of MCC347. Because the genomic integration site (the *PTPRG* locus on chromosome 3p14) is mapped for these tumors, we performed Southern blotting with flanking human sequence probes to examine cellular monoclonal integration. NheI-SacI digestion of MCC347 and 348 is predicted to generate a 3.1-kb fragment from the wild-type allele and a 3.9-kb fragment from the allele containing the integrated MCV DNA. Hybridization with a flanking human *PTPRG* sequence probe revealed that the 3.9-kb allele was present in MCC347 and 348 DNA but not in control tissue DNA (Fig. 3B). As predicted, the same fragment hybridized to a MCV T antigen sequence probe, consistent with both cellular and viral monoclonality in this tumor. These results provide evidence that MCV infection and genome integration occurred in this tumor before clonal expansion of tumor cells. MCV in MCC may have some parallels to high-risk human papillomavirus (HPV), which causes cervical cancer mainly after viral episome disruption and integration into the cervical epithelial cell genome (*16*).

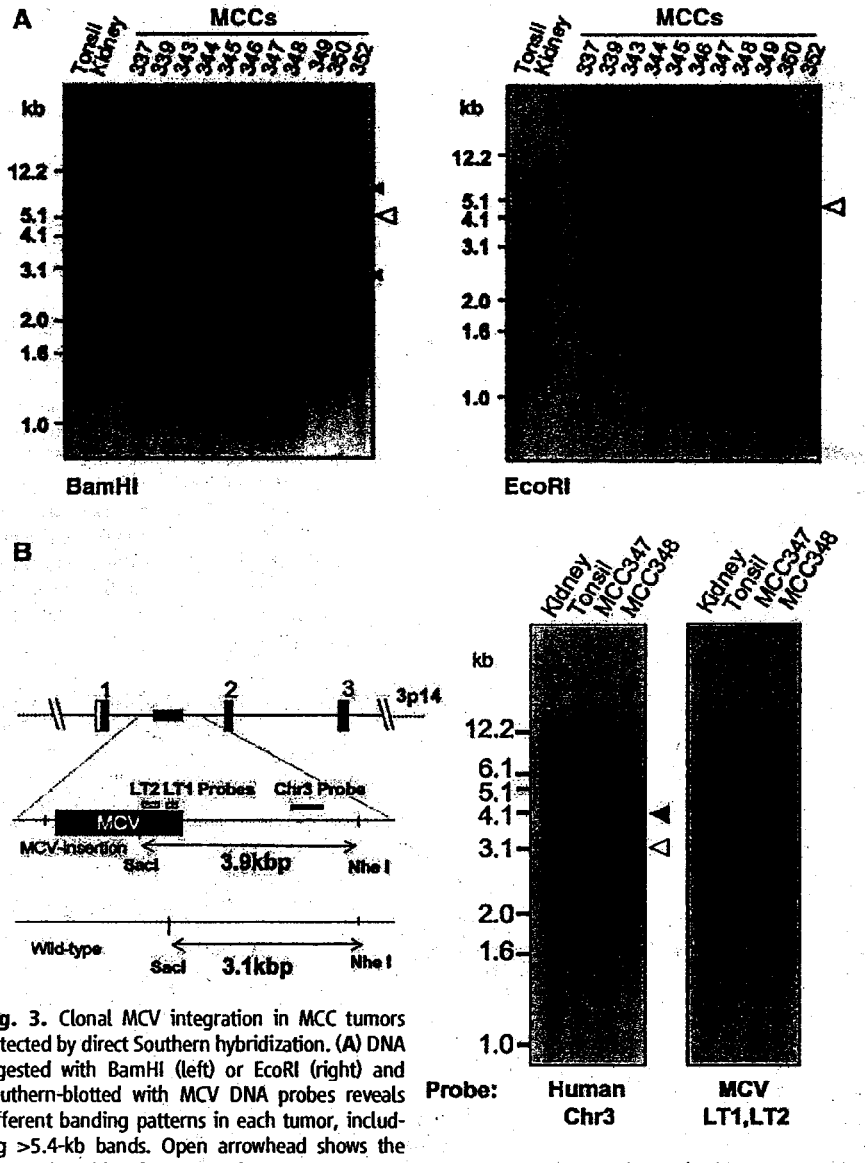

Fig. 3. Clonal MCV integration in MCC tumors detected by direct Southern hybridization. (**A**) DNA digested with BamHI (left) or EcoRI (right) and Southern-blotted with MCV DNA probes reveals different banding patterns in each tumor, including >5.4-kb bands. Open arrowhead shows the expected position for MCV episomal or concatenated-integrated genome (5.4 kb) with corresponding bands present in tumors MCC344 and 350. Tumors MCC339, 345, 347, 348, and 349 have different band sizes and doublet bands (solid arrowheads), consistent with genomic monoclonal integration. MCC352 has a prominent 5.4-kb band as well as higher and lower molecular weight monoclonal integration bands (BamHI), consistent with an integrated concatemer. Tumors MCC337, 343, and 346 have no MCV DNA detected by Southern blotting [bands at 1.5 kb (kidney) and 1.2 kb (MCC346) are artifacts]. (**B**) Viral and cellular monoclonality in MCC347 and 348. Tumor MCC347 and its metastasis MCC348 were digested with SacI and NheI and Southern-blotted with unique human flanking sequence probe [Chr3 (red), left] or viral probes [LT1 and LT2 (yellow), right]. The wild-type human allele is present in all samples at 3.1 kb (left). The MCC tumors, however, have an additional 3.9-kb allelic band formed by MCV DNA insertion into chromosome 3p14. Hybridization with probes for MCV T antigen sequence (yellow, right) generates an identical band.

If MCV plays a causal role in tumorigenesis, it could conceivably do so by several mechanisms, including T antigen expression, insertional mutagenesis, or both. Our DTS results show tumor expression of MCV T antigen, which has conserved DnaJ (*4*), pocket protein-binding LXCXE (*17*), and pp2A-binding (*18*, *19*) domains previously shown to play roles in polyomavirus-induced cell transformation. Mutational disruption of the *PTPRG* gene, which is suspected to be a tumor suppressor (*20*), could also play a role in MCC, although our Southern blot data suggest that MCV integration occurs at various genomic sites in different MCC tumors.

Our study validates the utility of DTS for the discovery of cryptic human viruses, but it has also revealed some limitations of the approach. Of the four tumors we sampled, only one (MCC347) was infected at high copy number. MCV transcripts in this tumor were present at 10 transcripts per million or about 5 transcripts per tumor cell. In future searches for other directly transforming tumor viruses (*21*), DTS should be used on multiple highly uniform samples sequenced to a depth of 200,000 transcripts or greater. Because DTS is quantitative, it is less likely to be useful in its current form for discovery of low-abundance viruses in autoimmune disorders or other chronic infectious diseases. Discovery of MCV by DTS nonetheless shows that DTS and related approaches (*22*) are promising methods to identify previously unknown human tumor viruses.

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Table 2. PCR for MCV DNA in comparison control tissues (n = 84). For detailed description of tissues and tissue sites, see table S2. MCV positivities marked with plus and minus symbols together are as in Table 1. For the various body site tissues, there were 59 samples; for the skin and skin tumor tissues, the sample size was 25 (table S2).

	MCV positivity
Various body site tissues	
Total MCV negative (%)	54/59 (92)
Total MCV positive (%)	5/59 (8)
Appendix control 1	−/+
Appendix control 2	−/+
Gall bladder	−/+
Bowel	−/+
Hemorrhoid	−/+
Skin and skin tumor tissues	
Total MCV negative (%)	21/25 (84)
Total MCV positive (%)	4/25 (16)
Skin	−/+
KS skin tumor 1	−/+
KS skin tumor 2	−/+
KS skin tumor 3	−/+

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Materials and Methods
Figs. S1 to S3
Tables S1 to S5
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Worldwide Human Relationships Inferred from Genome-Wide Patterns of Variation

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Human genetic diversity is shaped by both demographic and biological factors and has fundamental implications for understanding the genetic basis of diseases. We studied 938 unrelated individuals from 51 populations of the Human Genome Diversity Panel at 650,000 common single-nucleotide polymorphism loci. Individual ancestry and population substructure were detectable with very high resolution. The relationship between haplotype heterozygosity and geography was consistent with the hypothesis of a serial founder effect with a single origin in sub-Saharan Africa. In addition, we observed a pattern of ancestral allele frequency distributions that reflects variation in population dynamics among geographic regions. This data set allows the most comprehensive characterization to date of human genetic variation.

In the past 30 years, the ability to study DNA sequence variation has dramatically increased our knowledge of the relationships among and history of human populations. Analyses of mitochondrial, Y chromosomal, and autosomal markers have revealed geographical structuring of human populations at the continental level (*1*–*3*) and suggest that a small group of individuals migrated out of eastern Africa and their descendants subsequently expanded into most of today's populations (*3*–*6*). Despite this progress, these studies were limited to a small fraction of the genome, to limited populations, or both, and yield an incomplete picture of the relative importance of mutation, recombination, migration, demography, selection, and random drift (*7*–*10*). To substantially increase the genomic and population coverage of past studies (e.g., the HapMap Project), we have examined more than 650,000 single-nucleotide polymorphisms (SNPs) in samples from the Human Genome Diversity Panel (HGDP-CEPH), which represents 1064 fully consenting individuals from 51 populations from sub-Saharan Africa, North Africa, Europe, the Middle East, South/Central Asia, East Asia, Oceania, and the Americas (*11*). This data set is freely available (*12*) and allows a detailed characterization of worldwide genetic variation.

We first studied genetic ancestry of each individual without using his/her population identity. This analysis considers each person's genome as having originated from K ancestral but unobserved populations whose contributions are described by K coefficients that sum to 1 for each individual. To increase computational efficiency, we developed new software, *frappe*, that implements a maximum likelihood method (*13*) to analyze all 642,690 autosomal SNPs in 938 unrelated and successfully genotyped HGDP-CEPH individuals (*14*). Figure 1A shows the results for $K = 7$; those for $K = 2$ through 6 are in fig. S1. At $K = 5$, the 938 individuals segregate into five continental groups, similar to those re-

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No. 4

医薬品 研究報告 調査報告書

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研究報告の概要

○海外帰国者とその妻におけるブルセラ症:*Brucella melitensis*のヒト-ヒト感染の可能性

ブルセラ症は世界では一般的な人獣共通感染症で、年間50万例以上のヒト感染症例がある。ブルセラ症の一次感染は、*Brucella*種に汚染された殺菌処理されていない乳製品の摂取によって起こるが、複数の報告で男性から女性パートナーへブルセラ症が伝播した可能性が示唆されており、それらの症例は性交渉による感染と考えられてきた。

症例1:64歳の日本人男性が、6週間続く発熱で1998年6月2日に都内の病院に入院した。過去1週間の激しい腰痛も訴えた。入院時の血液培養からグラム陰性桿菌が検出され、*Brucella melitensis*バイオタイプ2と同定された。ブルセラ菌抗体価は800IUで、骨髄と肝生検からブルセラ症と確定された。患者は同年3月にイラクのバグダッドに10日間の滞在歴があり、滞在中にヒツジのチーズを摂取したことが判明した。抗生物質の投与によって症状は治まり、4ヵ月の投薬で完全に回復した。

症例2:患者1の妻で60歳の日本人女性が、1998年5月31日から発熱と左胸鎖関節の痛みを訴え始めた。血液と関節液の培養で*B melitensis*が生育した。ブルセラ菌抗体価は800IUであったが、抗生物質の投与によって回復した。患者はイラクへの渡航歴はなく、ブルセラ症に関する他のリスク要因もなかった。

考察:イラクを含め中東ではブルセラ症の発生数は多いが、日本では稀なことから、患者1は海外滞在中にブルセラ症に感染したと考えられる。2人の患者の発症には1ヵ月程度の間隔があり、標準的なブルセラ症の潜伏期間と一致する。患者1はイラクから日本に乳製品を持ち込んでおらず、患者2とブルセラ症との疫学的関連はない。患者1は疾患初期に患者2と性交渉があったことを報告しており、おそらく患者1から患者2への性感染が起こったと考えられる。同様に性感染と考えられる症例は過去にも報告されている。

報告企業の意見	今後の対応
イラクからの帰国者からその妻へ、ブルセラ症が性感染した可能性があるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、問診で発熱などの体調不良者を献血不可としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

2

Brucellosis in a Returned Traveler and His Wife: Probable Person-To-Person Transmission of *Brucella melitensis*

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Brucellosis is the most common zoonosis worldwide, with more than 500,000 new human cases annually. Although brucellosis is primarily transmitted to humans through the consumption of unpasteurized dairy products contaminated with *Brucella* species, several reports have indicated that brucellosis may be transmitted from a man to his female partner. It has been suggested that sexual intercourse is a means of transmission in these cases. Here, we describe an additional case of probable person-to-person transmission of *Brucella melitensis* in an elderly couple.

Case Report 1

A previously healthy 64-year-old Japanese man with a 6-week history of febrile illness was admitted to hospital in Tokyo, Japan, on June 2, 1998, following a 10-day visit to Baghdad, Iraq, on March 8, 1998. He also complained of severe lower back pain for 1 week. Findings on admission were fever (maximum temperature, 39.5°C) and normal pulse rate (80 beats/min). Neither heart murmurs nor adventitious breath sounds was heard. The liver was palpable 2 cm below the right costal margin; yet, the spleen was not palpated. He had tenderness of the lumber spine without abnormal neurological findings. He had no signs of epididymoorchitis. The white blood cell count was 8,400/μL and hemoglobin concentration 12.5 g/dL. Liver function tests showed elevation of alkaline phosphatase (378 IU/L) and alanine aminotransferase (67 IU/L). The erythrocyte sedimentation rate was 67 mm/h. Urinalysis findings were normal. Chest X-ray showed no opacities. T1-weighted magnetic resonance imaging of the spine revealed decreased signal intensity in the L3, L4, and L5 vertebral bodies and adjacent epidural space. These findings indicated that the patient had spondylitis, complicated by an epidural abscess.

The Gram-negative bacilli yielded by the blood culture at admission were subsequently confirmed as *Brucella melitensis* biotype 2. The *Brucella* antibody titer by the tube agglutination test was 800 IU. In addition, bone marrow and liver biopsy specimens showed evidence of granulomas consistent with brucellosis. A detailed travel history revealed that he had consumed sheep's cheese during his stay in Iraq. After confirmation of brucellosis, he was treated with intramuscular streptomycin (1 g daily), oral doxycycline (100 mg twice daily), and rifampicin (600 mg daily) for 1 month, and the fever and lower back pain gradually subsided. This treatment was followed by oral rifampicin (600 mg daily), trimethoprim–sulfamethoxazole (two standard-strength tablets twice daily), and tosufloxacin (200 mg thrice daily) for 4 months, with complete resolution.

Case Report 2

The wife of patient 1, a previously healthy 60-year-old Japanese woman, began to complain of fever and pain in the left sternoclavicular joint on May 31, 1998. Cultures of blood and the joint fluid grew *B melitensis* biotype 2. The *Brucella* antibody titer by the tube agglutination test was 400 IU. She was successfully treated with oral rifampicin (600 mg daily) and doxycycline (100 mg twice daily) for 6 weeks in combination with intramuscular streptomycin (750 mg daily) for the first 3 weeks. She did not visit Iraq with her husband and had no other risk factors for brucellosis.

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Discussion

The Middle East, including Iraq, has the highest incidence of brucellosis in the world, whereas Japan is considered to be a brucellosis-free country.[1] Brucellosis is one of the reportable infectious diseases in Japan. According to the national surveillance data, only three cases of human brucellosis and two of livestock brucellosis were reported between 1999 and 2005 in Japan. No outbreaks of animal or human brucellosis were reported in Japan in 1998. Considering the incubation period of brucellosis (usually 2–4 wk, up to several months), his consumption of sheep's cheese in a brucellosis-endemic country, Iraq, and the rarity of brucellosis in his residential country, Japan, it is likely that patient 1 contracted brucellosis during his stay abroad.

The serial interval of the disease onset between patient 1 and patient 2 was approximately 1 month, which is similar to the mean incubation period of human brucellosis. Although the incubation period of brucellosis varies widely, it is difficult to argue that a common source exposure, such as food poisoning, occurred in these two patients, since patient 1 did not bring any dairy products or animals into Japan from Iraq. Furthermore, patient 2 had no other epidemiological links to brucellosis. Therefore, it is strongly suggested that the disease was transmitted from patient 1 to patient 2.

Through a PubMed search (1966–2005), we found six case reports of probable person-to-person transmission, excluding cases associated with blood transfusion, bone marrow transplantation, and breast-feeding (Table 1). Two of them are associated with international travel. In summary, it seems that men with symptoms of brucellosis are able to transmit the disease to their female partners. It is speculated that sexual transmission occurred in these cases since this is well known in animals. Interestingly, Mantur and colleagues reported that *B melitensis* was isolated from the semen, urine, and saliva of a man with epididymoorchitis, who transmitted the disease to his wife.[6] However, the presence of epididymoorchitis does not seem to be related to the transmissibility of human brucellosis. Furthermore, another report described that *B melitensis* was isolated from the sperm of one patient.[8] Patient 1 reported that he had intercourse with patient 2 during the initial stages of the disease. Therefore, we consider that person-to-person transmission, probably sexual transmission, of *B melitensis* occurred in our case.

Table 1 Published case reports of probable person-to-person transmission of brucellosis between men and women (English literature only)

Case reports	Goossens et al[2]	Stantic-Pavlinic et al[3]	Ruben et al[4]	Lindberg et al[5]	Mantur et al[6]	Thalhammer et al[7]	Present case
Age (y), sex, risk factor of primary case	25, male, laboratory exposure	34, male, laboratory exposure	61, male, laboratory exposure	35, male, travel to endemic area	30, male, animal exposure	25, male, travel to endemic area	65, male, travel to endemic area
Epididymoorchitis	Absent	Absent	Absent	Present	Present	Absent	Absent
Country where primary case was infected	Belgium	Yugoslavia	United States	Spain	India	Syria	Iraq
Age (y), sex, relationship of secondary case	21, female, fiancée	30, female, spouse	61, female, spouse	30, female, girlfriend	22, female, spouse	ND, female, girlfriend	60, female, spouse
Serial interval between two cases	3 mo	3 mo	8 mo	5 mo	1 mo	2 mo	1 mo
Isolated *Brucella* species and biotype	*Brucella melitensis* biotype 3	*B melitensis* biotype 2	*B melitensis* biotype 3	*B melitensis* biotype ND	*B melitensis* biotype 1	*Brucella abortus* biotype ND	*B melitensis* biotype 2
Suspected transmission route	Sexual intercourse	Sexual intercourse	Sexual intercourse	Sexual intercourse	Sexual intercourse	Sexual intercourse	Sexual intercourse

ND = not determined.

Although it has a little role in the epidemiology of brucellosis, person-to-person transmission is rather important in areas where brucellosis is not endemic such as most of developed countries; brucellosis has become a common imported disease in these areas.[9] Febrile-returned travelers should be educated to abstain from sexual intercourse because they could transmit the diseases to their partners. We would like to add brucellosis to the list of travel-related infections that are transmissible through sexual intercourse. This unusual mode of transmission of a common zoonosis requires special attention.

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Declaration of Interests

The authors state that they have no conflicts of interest.

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医薬品 研究報告 調査報告書

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研究報告の概要

○2003年のアルジェリアにおける50年ぶりのペスト再興
2003年6月から7月にアルジェリアOran地区においてペストの集団感染が発生した。アルジェリアでは、この疾患が50年以上報告されていなかった。腺ペスト症例18名が特定され、*Yersinia pestis*が6名から分離された。初発患者を除き、全員が回復した。標的予防的化学療法、公衆衛生、ベクターコントロールが、感染制御上重要な役割を果たした。疫学的、分子生物学的な知見から、当該期間中、現地の保菌動物の存在が強く示唆されたが、その起源(再興または再持ち込み)については特定できなかった。主要な貿易港における、今回の突然かつ予期せぬペスト再興は、国際的に重要な意味を持つ公衆衛生問題の典型的な例である。また、今回の再興は、ペスト再興の危険性が現在確認されているnatural foci(げっ歯類がペスト菌を保有する地区)に限られるものではないことも示している。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2003年6月から7月にアルジェリアOran地区において、50年ぶりに腺ペストの集団感染が発生したとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き情報の収集に努める。

Plague Reappearance in Algeria after 50 Years, 2003

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An outbreak of plague occurred in the region of Oran, Algeria, from June to July 2003. Algeria had not reported this disease for >50 years. Eighteen bubonic cases were identified, and *Yersinia pestis* was isolated from 6 patients. Except for the index case-patient, all patients recovered. Targeted chemoprophylaxis, sanitation, and vector control played a crucial role in controlling the outbreak. Epidemiologic and biomolecular findings strongly suggested the existence of a local animal reservoir during this period, but its origin (resurgence or re-importation) could not be determined. This sudden and unexpected reemergence of plague, close to an important commercial seaport, is a textbook illustration of a public health event of international importance. It also demonstrates that the danger of plague reoccurrence is not limited to the currently indexed natural foci.

Plague is primarily a bacterial zoonosis affecting rodents. It is caused by *Yersinia pestis* and is transmitted from animal to animal by fleas. Humans usually become infected through the bite of an infected rodent flea. Bubonic plague, a severe infectious disease which, in the absence of appropriate antimicrobial drug therapy, can evolve to a rapidly fatal septicemia or pneumonia, can develop. A pneumonia form, which enables direct transmission to contacts, can be responsible for highly lethal outbreaks.

Currently, plague natural foci persist in Asia, the Americas, and Africa (where most human cases occur) (*1*). Plague foci have previously existed in the northern part of Africa but gradually disappeared in the last century, for unknown reasons. Libya is the only north African country that has experienced human cases in the past 40 years (*2*). In Algeria, archives report epidemics of plague as far back as the 14th century. These epidemics mainly affected ports, particularly that of Oran in 1556 and 1678 (3,000 deaths). In 1899, after an absence of nearly 100 years, plague reappeared in the port of Philippeville (now Skikda). Three large epidemics were subsequently reported in 1921 (185 cases), 1931 (76 cases), and 1944 (95 cases) as well as 158 sporadic cases. All but 2 cases occurred in ports (*3*,*4*). No natural focus of plague had ever been described in Algeria (*5*). We describe an outbreak of bubonic plague that occurred in 2003 in Algeria, where the last reported human case occurred in Oran in 1946 (*6*).

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Methods

During June 9–18, 2003, several patients with signs of severe infection and painful inflammatory adenopathy were admitted to the University Hospital of Oran. All came from Kehailia (35°29′N, 0°32′E), a village of 1,300 inhabitants 25 km south of Oran. After eliminating all other possible differential diagnoses, clinicians suspected plague. The diagnosis was confirmed on June 18 by results of analysis of a bubo (lymph node) aspirate. A technical crisis committee was set up, and a case definition was adopted (Table). Any patient with a febrile syndrome and adenopathy who resided in the prefecture of Oran was hospitalized.

Clinical samples collected from patients (blood, bubo aspirate, cerebrospinal fluid) were sent to the Microbiology Department, University Hospital, Oran. Several of the initial cases were first diagnosed with the rapid diagnostic test (RDT) for plague developed by the Institut Pasteur (*7*); however, all samples were also examined with standard bacteriologic methods. Direct examination of smears was performed after Wayson and Gram staining. Blood samples were cultured in Castaneda medium for at least 10 days

Table. Plague case definition adopted by technical crisis committee, 2003 plague outbreak, Oran region, Algeria*

Case definition	Criteria
Suspected	Clinical and epidemiologic characteristics compatible with plague; or, observation of suspect microorganisms on direct examination of clinical samples
Probable	Suspected case with anti-F1 antibodies in patient's blood; or, suspected case with a positive RDT without isolation of *Yersinia pestis* or in the absence of other cases reported in a radius of 10 km around the case
Confirmed	Culture positive for *Y. pestis*; or, RDT positive and *Y. pestis* isolated from patients living in a radius of 10 km around the case

*RDT, rapid diagnostic test.

at 28°C and examined daily. Suspected samples were inoculated into brain heart infusion and peptone broth and streaked on blood agar and cefsulodin-irgasan-novobiocin (Merck, Rahway, NJ, USA) plates. All media were incubated at 28°C. Bacterial identification was conducted with API 20 E strips (Analytab Products, Syosset, NY, USA) or individual tests in tubes. The biovar was determined (*8*). Antimicrobial drug susceptibility testing (ampicillin, amoxicillin-clavulanic acid, cefazolin, cefotaxime, gentamicin, amikacin, sulfamethoxazole, doxycycline) was conducted according to the technique of the Clinical and Laboratory Standards Institute (www.clsi.org). The serodiagnosis was determined by the ELISA-F1 technique (*9*). Serum samples from 30 study participants who had not contracted the disease but lived in the same area as the patients were used to determine the positive threshold of the technique. A serum was regarded as negative if its optical density at 490 nm (OD_{490}) was lower than a threshold defined as the mean (M) OD_{490} value of normal sera + 3 standard deviations (SD): $OD_{490} < M + 3$ SD. Sera with OD higher than this threshold were regarded as weak when the ratio $R = OD_{490}/(M + 3SD)$ was <2 and positive if R was ≥2.

Results

On June 9, 2003, a 19-year-old shepherd living in Kehailia was hospitalized with signs of septic shock (patient no. 2) (online Appendix Table, available from www.cdc.gov/EID/content/13/10/1459-appT.htm). He had been treated at home unsuccessfully with cephalosporins for inguinal adenopathy and fever during the previous 8 days. In the same village, 6 similar cases (nos. 3–8) occurred in the following days, until the diagnosis of plague was suspected and confirmed on June 18, first by RDT and then by isolation of a bacterium that had all the characteristics of *Y. pestis* biovar Orientalis and was susceptible to the antimicrobial agents tested. The epidemiologic investigation uncovered the index patient (no. 1), an 11-year-old child from Kehailia who was a cousin of case-patient 2. On June 2, an inguinal adenopathy with fever developed, and patient 2 was transferred to the hospital. He died 3 hours later, without a precise diagnosis.

Following the sanitation measures (reduction of rodent harborage, garbage removal, and vector control) implemented in Kehailia, no new cases of plague were reported in this locality after June 17. On June 19, a woman living in the suburbs of Oran (Hai Oussama) was hospitalized with bubonic plague (patient 9). The investigation showed that she had gone to Kehailia in the preceding days to consult a healer. Five cases of bubonic plague (nos. 10, 11, 14, 15, and 17) subsequently occurred from June 21 to July 16 among persons living in villages around Kehailia.

On June 28, a farmer and his wife (patients 12 and 13) who resided in Ain Temouchent, 50 km west of Kehailia (Figure), were hospitalized in Oran for symptoms suggestive of plague. The patients reported that they had not left their farm during the weeks preceding their illness. On July 1, a child from Beni Saf, on the Mediterranean coast 100 km southwest of Kehailia (Figure), had clinical signs of bubonic plague and a positive RDT result (patient 16). Neither he, nor his parents, had gone to the area of Kehailia or Ain Temouchent during the previous days. The last case (patient 18) occurred on July 22. The patient, a hunter who lived in Oran, had walked in the forest of M'sila, 30 km northwest of Kehailia, a few days before onset of his clinical signs.

Altogether, 18 cases were identified June 4–July 22, 2003: 10 confirmed, 3 probable, and 5 suspected (or 12 confirmed, 2 probable, and 4 suspected, according to the new World Health Organization case definition [*1*]). Most of the patients lived in unsanitary conditions, in close contact with livestock, and in the vicinity of storage areas of grain and fodder. In Kehailia, all the case-patients resided in different dwellings located within a 200-m radius. None of them reported direct contact with rodents. Sixteen of the 18 patients had an inguinal bubo, indicative of a flea bite on the leg. A septicemic form of plague developed in patients 1 and 2. Patient 1 died very soon after hospital admission. Patient 2 was admitted with a severe fever and neurologic syndrome and fell into a deep coma, despite broad-spectrum antimicrobial drug treatment that included vancomycin, cefotaxime, and gentamicin. He recovered from the coma 48 hours after treatment with ciprofloxacin (500 mg 2×/d for 30 days) was completed (F. Razik et al., unpub. data). No case of secondary pulmonary dissemination was observed. Other plague patients were treated with either doxycycline for adults (200 mg/d for 10 days) or cotrimoxazole for children (40 mg/kg/d for 10 days). All recovered without sequelae.

On the whole, 60 bubo aspirates, 143 blood samples, 6 sputum samples, and 2 cerebrospinal fluid samples were analyzed. In 5 samples, smear stains suggested infection

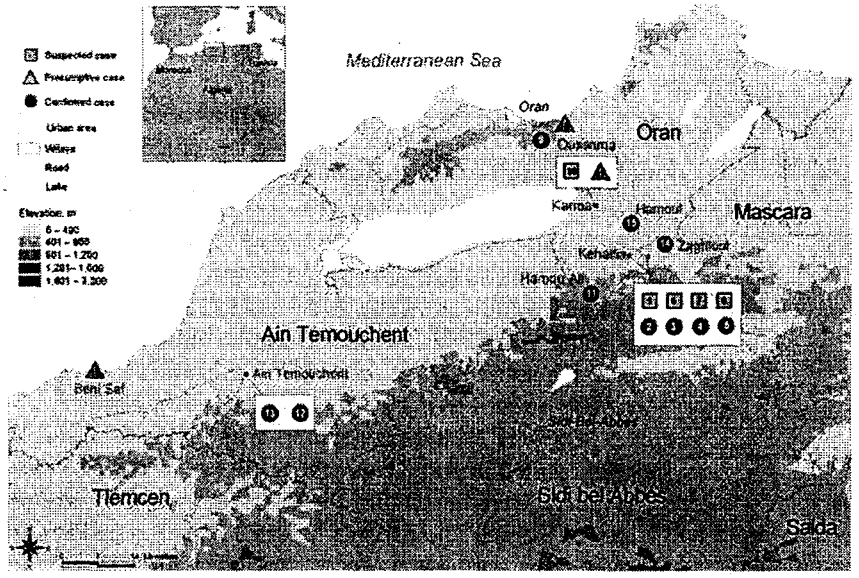

Figure. Geographic distribution of plague cases, Oran region, Algeria, June–July 2003. Boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization (WHO) concerning the legal status of any country, territory, city, or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. Data source: Ministry of Health Algeria. Map production: Public Health Mapping and GIS, Communicable Diseases, WHO. Copyright WHO, 2006. Used with permission.

with *Y. pestis* (online Appendix Table). Among the 18 patients, 12 had a positive RDT result, but *Y. pestis* was isolated from only 6 patients: 5 from bubo aspirates and 1 from the blood culture of a patient whose bubo was too small to be punctured (patient 13). Results of ELISA-F1 serologic test conducted on the serum samples from 15 of the 18 patients were strongly positive 3 times and slightly positive 3 times (online Appendix Table).

Discussion

Epidemiologic investigation did not identify any other plague patients before patient 1. It is unlikely that other cases occurred and remained undetected during this period since plague, even in its bubonic form, is a severe infection with high fatality rates.

For the first time, the RDT was used in an epidemic situation outside of Madagascar, where it was developed. The case definition had to take into account this particularity. The bacteriologic diagnosis is a long procedure (at least 4 days) and, in this epidemic context, RDT contributed to the effectiveness of the response. Of the 44 RDTs that were conducted, 12 had positive results; by contrast, culture was positive only for 6. Among the 15 patients for whom a serologic test was conducted (online Appendix Table), a specific antibody response developed only in 6. This absence of specific antibodies can be explained by the fact that serum specimens were taken before the appearance of anti-F1 immunoglobulin G, or by a rapid administration of antimicrobial drugs, which stopped development of an immune response. The 3 clearly seropositive patients were those from whom a positive culture was obtained.

The outbreak occurred in a poor rural settlement, with inadequate sanitation. The residents observed an increase in the population of commensal rodents, which is often associated with the harvesting period, but no unusual rodent mortality was noted during the weeks preceding the outbreak. The appearance during the same week of 2 new cases in Ain Temouchent (50 km west of Kehailia) and then 1 case in Beni Saf (100 km southwest of Kehailia) could not be explained. Nonetheless, the fact that the *Y. pestis* strains isolated in Kehailia and Ain Temouchent had identical pulsotypes (V. Chenal-Francisque et al., unpub. data) argues for a single focus and not for independent foci that emerged simultaneously.

A crisis committee designed and supervised a control strategy based on standardized case management, prophylactic treatment and follow-up of contacts sharing the same dwelling as plague patients, and vector control. Environmental sanitation measures in Kehailia contributed to reduction in the occurrence of new cases in this village. Intra- and peridomestic spraying with permethrin was conducted. Deltamethrin was dusted on the tracks and around the burrows of rodents located in a radius of 10 km around the dwelling of the patients. Uncontrolled killing of rats was prohibited.

No natural focus of plague had ever been described in Algeria. Past cases were always regarded as imported through the ports. The reappearance of human cases in this area can be explained in 2 ways: a recent importation of infected animals or a sudden manifestation of a natural focus that had remained silent for decades. It is noteworthy that Kehailia, the epicenter of the outbreak, is in the vicinity of flour mills built 4 years before the outbreak. These mills are supplied regularly with cereals by trucks arriving from the port of Oran. A part of this traffic was still run by railway a year before the outbreak, and a marshalling yard was installed a few kilometers from Kehailia. In 1919, this mode of importation was responsible for the plague outbreak that